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Osteoinductive potential of human demineralised bone and a bioceramic in the abdominal musculature of the rat*

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INTRODUCTION

The demonstration that both demineralised human bone preparations and bioceramics are capable of accelerating the healing of bone defects has led to the commercial development of such materials. Recently an investigation of the osteogenic potential of human demineralised bone preparations has shown that the method of preparation significantly affects the amount of new bone formation (Marinak, Mellonig & Towle, 1989). It is therefore important to examine commercially available tissue bank bone (demineralised, lyophilised and sterilised with ethylene oxide). In the past intramuscular implant sites have been used extensively to test both bone (mineralised and demineralised) and bioceramics (Urist, Jurist, Dubuc & Strates, 1970; Salama, Burwell & Dickson, 1973; Chalmers, Gray & Rush, 1975; Thorogood & Gray, 1975; Nade & Burwell, 1977; Yamazaki, Oida, Akimoto & Shioda, 1988). It has been argued that the advantage of an extraskeletal muscle implant site is that it minimises the contribution of mineralised tissues (and so provides a true indication of the osteoinductive potential of a given material) yet provides a milieu akin to that found in bone defect sites (Marinak *et al.* 1989). Using an intramuscular rodent model system similar to that described by Linden (1975), samples provided from a tissue bank were evaluated for osteogeneic potential. Using the same system a composite bioceramic of tricalcium phosphate and hydroxyapatite was assessed for any bone inductive capacity and bioresorbability.

MATERIALS AND METHODS

Seventeen Sprague–Dawley rats (ten females and seven males) were used in the following procedures. Two implant materials were used, human demineralised cortical bone chips (sized between 1–2 mm, supplied by the Pacific Coast Tissue Bank, Los Angeles, USA) and bioceramic granules, consisting of 80% tricalcium phosphate and 20% hydroxyapatite (sized between 1·4–2·5 mm, Ostilit® R3, Howmedica, London, UK). Ostilit factor R3 was selected for implantation because the manufacturers claimed that in clinical trials it was found to be resorbed in three months.

Rats were anaesthetised and, using a sterile technique, pouches were formed in the abdominal wall musculature by a small incision (approximately 5 mm long) in the lateral superficial muscle layer. Using fine forceps, the muscle layers were separated

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along the plane of the fibre bundles. Demineralised bone chips, which had been soaked previously for one hour in normal saline, were placed in muscle pouches on one side of the linea alba (one chip per pouch). An Ostilit granule was placed in each muscle pouch on the opposite side. In the twelve animals in this group up to six pouches were made, three on each side of the linea alba. In another group of five animals, granules were implanted in close contact with bone chips in the same pouch. Pouches were closed with non-absorbable suture. At thirty, sixty and ninety days post-operatively groups of six, five and six rats respectively were killed by cervical dislocation and the implants, together with approximately 1 cm^2 of surrounding muscle, were excised. The specimens were fixed in formal saline (10%) and embedded in Rallwax. Interrupted serial sections of 7 μm thickness were cut and stained with haematoxylin and eosin. The histological response to the implants over a period of three months was examined and representative photographs were taken. The size of the implants was assessed radiographically at each of the three time points (30, 60 and 90 days) and from the histological sections using an ocular graticule fitted to a Leitz Diaplan microscope. From each sample, sections which had the largest implant diameter were selected for measurement. At least six sections were measured for each sample, and eight samples assessed at each time point.

RESULTS

Ostilit implants

There was no large scale inflammatory reaction around the implant; it was simply encapsulated within a sheath composed of elongated fibroblastic cells arranged in a layer of between four to eight cells thick. Beneath this sheath multinucleated giant cells were noted. These features were consistently observed throughout the time points examined and there was little change with time (Fig. 1 *a–c*). The cells encapsulating the implant were tightly apposed to the irregular contours of the implant surface, although there was no cellular penetration into the implant at any time point throughout the ninety day period. The staining of the implant was less intense at the periphery and this might be taken to represent a small degree of resorption. However this pale zone did not increase in size with time. Similarly, the granule size was found not to have altered when examined by both histological measurement and radiographical assessment. Measurements taken from interrupted serial sections showed a mean granule size of 1·91 mm, 1·52 mm and 1·96 mm at 30, 60 and 90 days respectively. Given that the initial granule size range was between 1·4–2·5 mm, these results show no substantial resorption of the granule during the three months period. It is interesting to note that the general tissue reaction surrounding the Ostilit implant was in all respects similar to that seen surrounding the proline suture (Fig. 1 *d*).

Demineralised bone

In contrast to the bioceramic granule, there was an extensive mononuclear cell inflammatory infiltrate around the bone chip and this infiltrate persisted throughout the period of examination (Fig. 2 *a–c*). These inflammatory cells extended into the central canals of the Haversian system. Multinucleate giant cells were only occasionally seen apposed to the periphery of the chip. Small, well-defined resorptive lacunae were noted; these may represent osteoclastic activity (Fig. 2 *b*). The osteocytic lacunae throughout the bone were empty at thirty days. By Day sixty however, some peripheral parts of the bone chip exhibited filled osteocytic lacunae with osteocytes visible (Fig. 2 *d*). At this stage large numbers of lymphocytes were still present at the periphery and resorptive bays representing osteoclast activity could be seen eroding the chip. At

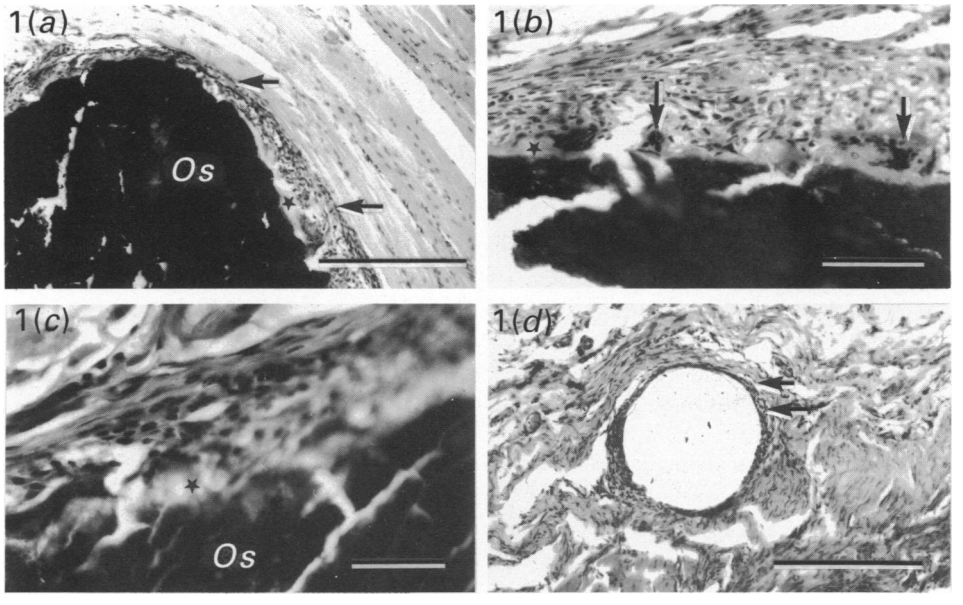

Fig. 1 (*a–d*). (*a*) Day 60 specimen showing Ostilit (*Os*) granule encapsulated within a fibroblastic sheath (arrows). Cracks within the particle represent a processing artefact. Bar, 0·5 mm. (*b*) Day 90 Ostilit granule with only few inflammatory cells and occasional multinucleate giant cells (arrow). Bar, 50 μm. (*c*) Day 60 individual Ostilit implant (*Os*) with surrounding fibroblasts. The less intensely staining rim of the Ostilit is apparent (★ in *a–c*). Bar, 20 μm. (*d*) Day 60 section demonstrating the non-absorbable suture used to close the muscle pouches. The fibrous encapsulation (arrows) is essentially similar to that around the Ostilit. Bar, 0·5 mm.

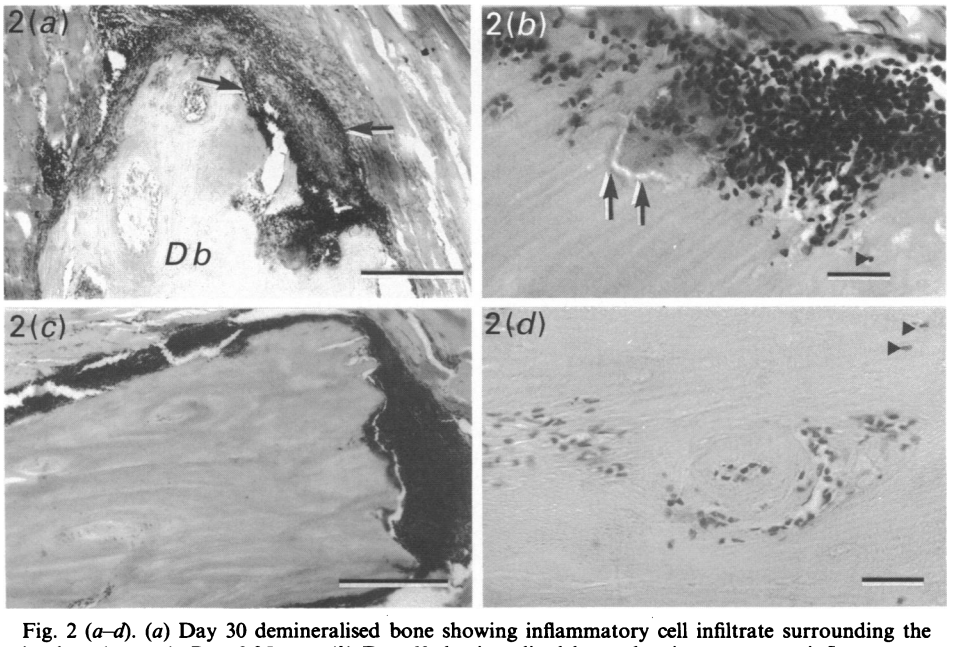

Fig. 2 (*a–d*). (*a*) Day 30 demineralised bone showing inflammatory cell infiltrate surrounding the implant (arrows). Bar, 0·25 mm. (*b*) Day 60 demineralised bone showing numerous inflammatory cells at the periphery of the implant. A resorptive bay (arrows) is seen with multinucleate giant cells present. An occupied osteocytic lacuna can be seen (arrowhead). Bar, 50 μm. (*c*) Day 90 demineralised bone showing persistence of the inflammatory cell reaction around the implant. Bar, 0·25 mm. (*d*) Day 60 demineralised bone showing filled osteocytic lacunae (arrowheads). Bar 40 μm.

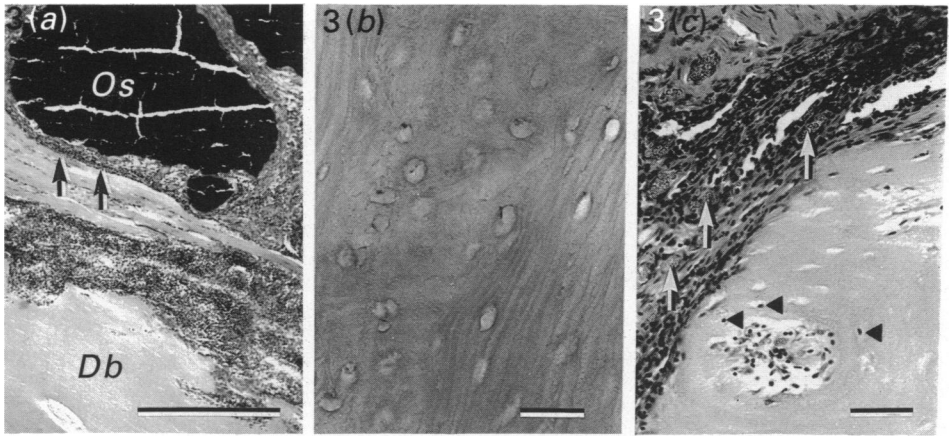

Fig. 3 (*a–c*). (*a*) Day 30 combined implants showing the Ostilit (*Os*) and demineralised bone (*Db*) in close apposition. There is an intense inflammatory cell infiltrate around the bone whereas the Ostilit is encapsulated in fibrous tissue (arrows). Bar, 0·5 mm. (*b*) Typical appearance of Day 30 demineralised bone showing empty osteocytic lacunae. Bar, 40 μm. (*c*) Day 60 combined implants showing that the demineralised bone is surrounded by a marked angiogenic response. Vessels are clearly seen (arrows). The inflammatory response is not as marked in intensity. Osteocytes are seen in lacunae (arrowheads). Bar, 100 μm.

ninety days there was a strong vascular response around the margins of the chip with early vascular ingress: new bone was apparent within several Haversian canals, but bone formation was not seen at the periphery of the bone chip.

Combined Ostilit and demineralised bone implants

In all the combined implants there were varying degrees of apposition, ranging from 20 μm to 770 μm (mean 227 μm, n = 8). The cellular response seen around both the Ostilit and bone implants was essentially similar to that seen around the individual granule or chip (Fig. 3*a*). Events around the Ostilit implant were identical in all respects and at all times to those around the isolated granule. For example, fibroblastic encapsulation, the less intensely stained rim and scattered multinucleate giant cells were all observed. In addition the granule size did not alter. Similarly the bone chips at the early time point were surrounded by large numbers of inflammatory cells which penetrated the cut lamellar ends of the bone. The osteocytic lacunae of the chip were empty (Fig. 3*b*), but Haversian canals showed cellular infiltration. At sixty days there was a marked angiogenic response with large numbers of capillary blood vessels in the connective tissue surrounding the bone chip (Fig. 3*c*). At this time there was a reduction in the number of inflammatory cells around the bone chip. Multinucleate giant cells were again observed in similar numbers to these around the isolated chip. At ninety days there was little change. An inflammatory cell infiltrate persisted, the chip had not been resorbed and although vascular invasion of the interstices had occurred, bone formation at these sites was no greater than that seen at 90 days in the isolated implant.

DISCUSSION

The results of this study have clearly shown that human demineralised bone, when implanted into a xenogeneic intramuscular site, has only limited osteoinductive capacity, and substantially confirmed the findings of Marinak *et al.* (1989). These

authors found that human bone implants demineralised by different preparative techniques, when implanted in a subcutaneous site resulted in sparse new bone formation. This may have reflected the choice of implant site, as it is well known that osteoinductive competence (measured in terms of positive results and bone yields), of a skeletal muscle site is better than subcutaneous tissue (Harakas, 1984). In the present study we have used an intramuscular site, but the new bone formation although not morphometrically assessed appeared less than that reported by Marinak *et al.* (1989) despite the period of study being twice as long. One consistent feature of the human bone in this site was a persistent inflammatory reaction which was not observed either with allografts implanted into a similar site (Linden, 1975) or indeed with xenografts (Marinak *et al.* 1989). Given that some authors have argued that osteoinduction is not species specific (Mulliken & Glowacki, 1980; Mulliken *et al.* 1981), this inflammatory response may be surprising and perhaps reflects greater retention of antigenicity of the bank bone. This is an area of controversy as there are conflicting reports on the value of xenografts in the clinical situation (Salama, 1982; Burwell, 1985). Furthermore the inclusion of ethylene oxide sterilisation could explain the low osteogenic potential of the bone as this agent is known to cross-link proteins.

The bioceramic had no observable effect either in isolation or in combination with the bone implant. It was not even resorbed within the three months period of the study. This lack of biodegradability conflicts with the manufacturers' claim that the material would have been resorbed within this period of time but supports recent studies which have shown that tricalcium phosphate is still present four months after implantation in a muscle site (Yamazaki *et al.* 1988). It is also known that hydroxyapatite is more insoluble than tricalcium phosphate (Burwell, 1985). Thus a combination of the two ceramics would be expected to show low bioresorbability. Our rationale for implanting a ceramic along with demineralised bone was that the material would act to guide bone formation from the implanted bone (osteoconduction) and thus a 'spillover' of osteogenic events would have occurred. This was not the case; our results indicated that the cellular events around the combined implants were in fact no different to those associated with the isolated bioceramic granule or bone chip. It would therefore appear that further analysis of both these commercially available implant materials is required to assess their value in the clinical situation.

SUMMARY

Human demineralised cortical bone chips ('bank' bone) and a particulate bioceramic (20% hydroxyapatite: 80% tricalcium phosphate) were implanted into muscle pouches in the rat abdominal wall. Combined implants of both bone and bioceramic were also made. The implants were examined histologically at thirty day intervals over a three month period. The results showed that the hydroxyapatite: tricalcium phosphate bioceramic was not resorbed and did not induce bone formation when implanted alone or in combination with human demineralised bone. In contrast the bank bone was osteogenic but, even at three months, bone formation was sparse. These results are in accord with recent findings that the use of xenogeneic model systems to evaluate the osteogenic potential of human bone is limited.

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